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Effects of feeding lauric acid or coconut oil on ruminal protozoa numbers, fermentation pattern, digestion, omasal nutrient flow, and milk production in dairy cows[1]

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ABSTRACT

The objectives of this study were to evaluate the feeding of coconut oil (CO), in which lauric acid (La) comprises about 50% of the fatty acid composition, as a practical rumen protozoa (RP) suppressing agent, to assess whether the source of La affects ruminal fermentation and animal performance and to test whether suppressing RP improves N utilization, nutrient digestion, nutrient flow at the omasal canal, and milk production. Fifteen multiparous Holstein cows (3 fitted with ruminal cannulas) and 15 primiparous Holstein cows (3 fitted with ruminal cannulas) were used in a replicated 3 × 3 Latin square experiment with 14 d of adaptation and 14 d of sample collection. Diets were fed as total mixed ration and contained (dry matter basis) 10% corn silage, 50% alfalfa silage, and 40% concentrate. The control diet contained 3% (dry matter basis) calcium soaps of palm oil fatty acids (Megalac, Church & Dwight Co. Inc., Princeton, NJ) as a ruminally inert fat source and had no added La or CO. Diets with La and CO were formulated to contain equal amounts of La (1.3%, dry matter basis). Dry matter intake was not affected by treatment. Both CO and La reduced RP numbers by about 40%. Lauric acid reduced yield of milk and milk components; however, CO did not affect yield of milk and yields of milk components. Both La and CO caused small reductions in total VFA concentration; CO increased molar proportion of ruminal propionate, reduced ruminal ammonia and branched-chain volatile fatty acids, suggesting reduced protein degradation, and reduced milk urea N and blood urea N concentrations, suggesting improved protein efficiency. Lauric acid reduced total-tract apparent digestibility of neutral detergent fiber and acid detergent fiber as well as ruminal apparent digestibility of neutral detergent fiber and acid detergent fiber as measured at the omasal canal; however, CO did not alter fiber digestion. Microbial protein flow at the omasal canal, as well as the flow of N fractions at the omasal canal, did not differ among treatments. Results from this experiment have confirmed that dietary La is not a practical agent for suppressing RP population in dairy cows, mainly because of its negative effects on fiber digestion and ruminal fermentation. Intake of CO appeared to reduce ruminal and improve protein efficiency, but did not improve milk production, milk composition, or increase microbial outflow from the rumen. Based on the results of this study, a 40% reduction of RP population is not sufficient to improve N utilization in dairy cows.
Key words: coconut oil, dairy cow, protozoa

INTRODUCTION

Protein is an expensive dietary nutrient; furthermore, excessive excreted N is an important environmental concern. Therefore, improving N utilization is a major challenge in ruminant nutrition research. The main sources of AA for ruminant animals are the microbial protein synthesized in the rumen and RUP; however, according to Jouany (1996), ruminal protozoa (**RP**) have a negative effect on protein utilization in ruminants because they reduce ruminal outflow of both microbial protein and RUP. Moreover, RP are the main contributors to bacterial protein turnover in the rumen (Wallace and McPherson, 1987), which is a consequence of RP predation on bacteria. Rumen protozoa possess protease (Forsberg et al., 1984), peptidase (Newbold et al., 1989), and deaminase (Itabashi and Kandatsu, 1975) activity; therefore, they actively degrade proteins, peptides, and AA, producing large amounts of ruminal ammonia, which they cannot completely use, therefore contributing to urinary urea excretion.

Medium-chain FA, such as lauric acid (**La**; C12:0), have been shown to have potent antiprotozoal effects (Newbold and Chamberlain, 1988; Hristov et al., 2011; Faciola and Broderick, 2013). Furthermore, they may

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be used routinely in farm operations and may have the potential to replace hazardous antiprotozoal chemicals.

Although suppression of RP may have the potential to improve N utilization in the rumen, achieving the level of RP suppression that would allow such improvement is still a challenge. Faciola et al. (2013) observed a strong antiruminal protozoa activity of La when a single dose of 160 g/d was given via ruminal cannula, reducing RP by 90% within 2 d of treatment. However, when La was fed at 160 and 240 g/d in the TMR, RP were reduced by only 25 and 30%, respectively, showing that these amounts mixed in the diet were not sufficient to suppress RP effectively. In a subsequent study, Faciola and Broderick (2013) tested greater doses of La in the TMR (240, 480, and 720 g/d), aiming to determine the dietary amount of La that would effectively suppress RP. These amounts reduced RP by 28, 50, and 64%, respectively; however, the 2 highest amounts also drastically reduced DMI and impaired ruminal fermentation and, consequently, decreased milk production. It has been speculated that high doses of La may affect diet palatability (Hristov et al., 2011). Coconut oil (**CO**), in which La comprises about 50% of the FA composition, may be an alternative to feeding La because it may not have the same negative effects on DMI and ruminal fermentation (Faciola and Broderick, 2013). Therefore, the main goal of the present study was to evaluate dietary CO as a practical RP-suppressing agent and to test whether suppressing RP improves N utilization, nutrient digestibility, nutrient flow at the omasal canal, as well as milk composition and production. We hypothesized that CO would suppress RP numbers without depressing DMI and ruminal fermentation, thereby improving N utilization and milk production.

MATERIALS AND METHODS

Care and handling of all experimental animals, including ruminal cannulation, were conducted under protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee. Fifteen multiparous Holstein cows (3 fitted with permanent ruminal cannulas), averaging 2.5 ± 0.9 parity, 71 ± 39 DIM, 39.8 ± 3.7 kg/d of milk, and 621 ± 60 kg BW, and 15 primiparous Holstein cows (3 fitted with permanent ruminal cannulas), averaging 123 ± 53 DIM, 36.3 ± 4 kg/d of milk, and 545 ± 62 kg BW at the beginning of the study, were blocked by parity and by DIM within parity into 10 squares of 3 cows (2 squares of cannulated cows). Cows were randomly assigned within squares to 3 balanced dietary treatment sequences [i.e., with each diet following every other diet twice in each pair (multiparous and primiparous) of squares over the trial] in a replicated 3 × 3 Latin square with 14 d of adaptation and 14 d of sampling.

Composition of the fermented feeds fed is in Table 1. Diets were fed as a TMR and contained (DM basis) 10% corn silage, 50% alfalfa silage, and 40% concentrate (Table 2). The control diet contained calcium soaps of palm oil FA (Megalac, Church & Dwight Co. Inc./Arm & Hammer Animal Nutrition, Princeton, NJ) as a ruminally inert fat source with no added La (99% La, KIC Chemicals Inc., Armonk, NY) or CO (Columbus Food Inc., Chicago, IL). The calcium soaps of palm oil FA, La, and CO were first thoroughly mixed with ground shelled corn and then mixed with the other concentrate ingredients before being mixed with the forages and fed as TMR. Both control and CO diets had the same ether extract content. Diets La and CO were formulated to contain equal amounts of La (1.3% on a DM basis), either as La (diet La) or in the form of coconut oil (diet CO).

All cows were injected every other week with bovine somatotropin (500 mg of Posilac, Elanco Animal Health, Greenfield, IN) from about 60 DIM; injections were synchronized such that animals received a full dose on d 1 and at 14-d intervals throughout the trial. Cows were housed in tiestalls and had free access to water during the trial.

Diets were offered once daily at 1000 h. Orts were collected and weights recorded at 0900 h and feeding rate was adjusted daily to yield orts of about 5 to 10% of intake. Weekly composites of corn silage, alfalfa silage, high-moisture shelled corn, TMR, and orts were taken from daily samples of about 0.5 kg that were stored at −20°C. Weekly samples were also taken of ground corn grain and solvent-extracted soybean meal and stored at room temperature. The DM was determined in weekly composites of corn silage, alfalfa silage, and rolled high-moisture shelled corn by drying at 60°C for 48 h and in weekly samples of ground corn grain and solvent-extracted soybean meal at 105°C, according to AOAC (1980). Weekly samples of feed ingredients were also analyzed for total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI). Ingredient DM and N contents were used to adjust dietary composition weekly to maintain constant DM proportions from each feed ingredient and equal CP contents in each diet. Intake of DM was computed based on the 60°C DM determinations for TMR and orts. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for total N (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI); DM at 105°C; ash and OM by AOAC (1980) methods; sequentially for NDF and ADF; and

Table 1. Chemical composition of fermented feeds

Item	Alfalfa silage	Corn silage	High-moisture shelled corn
DM, %	38.4	37.5	73.5
CP, % DM	21.5	6.9	7.3
NDF, % DM	37.7	42.3	8.4
ADF, % DM	28.5	21.1	2.3
Ash, % DM	11.1	4.0	1.9
NDIN, % total N	7.1	5.8	7.0
ADIN, % total N	3.2	1.1	3.9
NPN, % total N	62.2	60.0	49.5
Free AA-N,[1] % total NPN	33.1	32.5	23.2
Ammonia N, % total NPN	6.5	8.8	3.7
Unidentified N[2] of total NPN	22.6	18.7	22.6
pH	4.7	4.2	4.4

[1]Computed based on a mean 40.3 μmol of total free AA/mg of N in alfalfa and corn silage.

[2]Unidentified N = total N – Free AA-N – Ammonia-N (Broderick, 1987).

Table 2. Composition of diets

Item	Control	Lauric acid	Coconut oil
Ingredient, % DM			
Alfalfa silage	50.0	50.0	50.0
Corn silage	10.0	10.0	10.0
High-moisture shelled corn	16.8	18.8	16.8
Dry molasses	9.9	9.9	9.9
Solvent soybean meal	4.6	4.3	4.6
Ground shelled corn	4.1	4.1	4.1
Calcium soaps of palm oil FA[1]	3.0	0.0	0.0
Lauric acid	0.0	1.3	0.0
Coconut oil	0.0	0.0	3.0
Sodium bicarbonate	0.75	0.75	0.75
Limestone	0.36	0.36	0.36
Dicalcium phosphate	0.24	0.24	0.24
Salt	0.16	0.16	0.16
Vitamin-mineral premix[2]	0.08	0.08	0.08
Composition			
DM, %	49.2	48.9	49.4
OM, % of DM	92.9	92.8	92.9
NDF, % of DM	28.4	28.7	28.4
ADF, % of DM	18.4	18.4	18.4
CP, % of DM	16.1	16.2	16.1
RDP,[3] % of DM	10.4	10.5	10.4
RUP,[3] % of DM	5.1	5.0	5.1
Ether extract, % of DM	6.9	5.5	6.9
Ca,[3] % of DM	1.35	1.06	1.06
P,[3] % of DM	0.37	0.37	0.37
NFC[4]	39.2	40.2	39.2
NE_L,[5] Mcal/kg of DM	1.65	1.64	1.65
NDIN, % of total N	6.30	6.32	6.30
ADIN, % of total N	2.58	2.59	2.58
Fraction B3,[6] % of total N	3.72	3.73	3.72
NPN,[7] % of total N	20.1	20.1	20.1

[1]Megalac; Church & Dwight Co. Inc., Princeton, NJ. Contained 85% fat.

[2]Provided (per kilogram of DM): 56 mg of Zn as zinc oxide, 46 mg of Mn as manganous oxide, 22 mg of Fe as ferrous sulfate, 12 mg of Cu as copper sulfate, 0.9 mg of I as potassium iodide, 0.4 mg of Co as cobalt oxide, 0.3 mg of Se as sodium selenite, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

[3]Predicted from the NRC (2001) model.

[4]NFC = 100 − (% NDF + % CP + % fat + % ash) + % NDIN × 6.25, according to NRC (2001) model and using fat contents of individual dietary ingredients from NRC (2001) tables.

[5]Computed by discounting dietary energy based on actual DMI (NRC, 2001).

[6]Fraction B3 (Fox et al., 2004) = NDIN (% of total N) – ADIN (% of total N).

[7]Proportion of total N soluble in 10% (wt/vol) TCA (Muck, 1987).

using a heat-stable α-amylase and Na_2SO_3 (Van Soest et al., 1991; Hintz et al., 1996) for indigestible ADF (ADF remaining after a 12-d in situ incubation; Huhtanen et al., 1994), NDIN and ADIN (N fraction in NDF and ADF residue, respectively), and ether extract (AOAC, 1990). Composite samples of TMR were also analyzed for NPN (Muck, 1987; Leco FP-2000 N Analyzer; Leco Instruments Inc.). Orts and TMR were analyzed for La as described by Sukhija and Palmquist (1988). For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

On d 21 of each period, about 100 to 200 mL of digesta was collected from 4 locations in the ventral rumen at 0 (just before feeding), 1, 2, 4, 8, 12, 18, and 24 h after feeding, strained through 2 layers of cheesecloth, and pH was measured immediately using a glass electrode. A daily composite of strained ruminal fluid was prepared for each cow at each treatment and RP counts were determined in duplicate as described by Dehority (1993). Two 10-mL samples of composite ruminal fluid were then preserved in scintillation vials by addition of 0.2 mL of 50% H_2SO_4 and stored at −20°C. Just before analysis, samples were thawed and centrifuged (15,300 × *g* for 20 min at 4°C) and flow-injection analyses (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI) were applied to supernatants to determine ammonia, using a phenol-hypochlorite method (Lachat Method 18-107-06-1-A; Lachat), and total AA, using a fluorimetric procedure based on the reaction with *o*-phthaldialdehyde (Roth, 1971). Leucine was the standard in the *o*-phthaldialdehyde assay and total AA are reported in Leu equivalents. Samples were also thawed and centrifuged (28,000 × *g* for 30 min at 4°C) for determination of individual and total ruminal VFA using a modification of the gas-liquid chromatography method for free FA as described by Brotz and Schaefer (1987). This method does not resolve isovalerate and 2-methylbutyrate, which are reported as isovalerate plus 2-methylbutyrate.

Omasal sampling was performed on the rumen cannulated cows during the last week of each period using the techniques developed by Huhtanen et al. (1997) and Ahvenjärvi et al. (2000), as adapted by Reynal and Broderick (2005), to quantify digesta flow out of the rumen. Indigestible NDF (Huhtanen et al., 1994), $YbCl_3$ (Siddons et al., 1985), and Co-EDTA (Udén et al., 1980), which are mainly associated with, respectively, the large particle, small particle, and fluid phases of digesta, were used as flow markers at the omasal canal. Indigestible NDF was determined in large particles, small particles, and TMR (but not in fluid phases; Ahvenjärvi et al., 2000). Samples (0.35 g) were weighted into duplicate 5 × 10-cm Dacron bags (Sericol, Broadstairs, UK) with a 6-μm pore size, incubated in the rumens of 2 cows for 12 d, rinsed with water, then subjected to NDF analysis as previously described. The external microbial marker, ^{15}N, was used to quantify microbial NAN flow from the rumen. The triple marker technique of France and Siddons (1986) was used to determine the proportions with which to recombine the 3 phases to produce omasal true digesta. Before marker infusion began, whole ruminal contents were taken from each cow to determine the background ^{15}N abundance. Cobalt-EDTA, $YbCl_3$, and $^{15}NH_4SO_4$ containing 10% atom excess ^{15}N (Isotec, Miamisburg, OH) were dissolved in distilled water and continuously infused into the rumen at rates of 2.01 g of Co, 2.88 g of Yb, and 210 mg of ^{15}N per day in 2.62 L/d of solution. Markers were continuously infused for 158 h from 0800 h on d 20 to 2200 h on d 26 using a peristaltic pump (AutoAnalyzer II, Technicon Corp., St. Louis, MO). After 86 h of infusion, omasal samples were collected at twelve 2-h intervals over a 3-d period to represent the 24-h day. Sampling protocols, including confirming that sample tubes were correctly positioned in the omasal canal, sampling times and volumes, sample processing, isolation of fluid- and particle-associated bacteria, digesta marker analyses, and preparation of omasal true digesta were as described by Reynal and Broderick (2005) and Brito et al. (2007), except that ammonia and protozoa were not isolated for determination of ^{15}N enrichment. Samples of omasal true digesta were analyzed for total N, DM (105°C), ash, OM, NDF, ADF, NDIN, and ADIN as detailed previously for feed samples. Samples of omasal true digesta and isolated bacteria were treated with K_2CO_3 (Brito et al., 2007) to remove residual ammonia and analyzed for total N (equivalent to NAN) and for ^{15}N abundance using a Costech 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) interfaced to a Thermo-Finnigan Delta-Plus Advantage isotope ratio mass spectrometer (Thermo-Electron GmbH, Bremen, Germany). Equations used to compute flows of nutrients of dietary and microbial origin and extents of ruminal digestion were those detailed by Brito et al. (2007).

Cows were milked twice daily at 0600 and 1700 h and milk yield was recorded at each milking in all experimental periods. Milk samples from morning and afternoon milkings were collected on d 18 and 25 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a spectrum analyzer (FT6000; Foss North America Inc., Eden Prairie, MN) using AOAC (1990) method 972.16. For MUN determination, 5 mL of milk from all milking samples were treated with 5 mL of 25% (wt/vol) TCA. Samples were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman

No. 1 filter paper (Whatman Inc., Piscataway, NJ). Filtrates were stored at −20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow injection (Lachat Quik-Chem 8000 FIA; Lachat Instruments). Concentrations and yields of fat, true protein, and lactose, as well as SNF and MUN concentration were calculated as weighted means from morning and afternoon milk yields on each test day. Yield of 3.5% FCM was calculated according to Sklan et al. (1992).

Efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk and FCM by mean DMI. Efficiency of utilization of feed N was calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake, assuming no net deposit or mobilization of N from body tissues.

On d 26 and 27 of each period, 2 spot urine and 2 spot fecal samples were collected from all 28 cows at 6 and 18 h after feeding. Fecal samples were dried in a forced-draft oven (60°C; 72 h) and then ground through a 1-mm screen (Wiley mill). Equal DM from each fecal subsample was mixed to obtain 1 composite sample for each cow in each period. Fecal samples were analyzed for total DM, ash, OM, N, NDF, ADF, and indigestible ADF as described earlier. Indigestible ADF was used as an internal marker to estimate both apparent total tract nutrient digestibility and fecal output (Cochran et al., 1986). Urine samples were acidified immediately after collection by diluting 1 volume of urine with 4 volumes of 0.072 N H_2SO_4 and stored at −20°C. Later, urine samples were thawed at room temperature and filtered through Whatman No. 1 filter paper. Filtrates were analyzed for creatinine using a picric acid method (Oser, 1965) adapted to flow-injection analysis (Lachat Quik-Chem 8000 FIA; Lachat Instruments), for total N using a N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc.), for allantoin using the method of Vogels and van der Grift (1970) adapted to a 96-well plate reader, for uric acid using a commercial kit (No. 683–100P, Sigma Chemical Co., St. Louis, MO), and for urea with the colorimetric method also used for MUN. Daily urine volume was calculated based on individual BW and using a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urinary urea N, total N, and purine derivatives (allantoin plus uric acid) were calculated based on their individual daily excretion multiplied by daily urine volume.

Blood samples were taken into heparinized tubes 4 h after feeding from the coccygeal artery or vein of each cow on d 26 of each period and stored at −20°C until analyzed. After thawing at room temperature, 5 mL of blood were transferred to a 15-mL centrifuge tube, 1.25 mL of 25% TCA (wt/vol) was added, and tubes were vortexed, held for 30 min at room temperature, centrifuged (15,000 × g, 15 min, 4°C), and the supernatants were stored at −20°C until analyzed for BUN with the flow injection system used for MUN.

Results were analyzed using the MIXED procedures of SAS (SAS Institute Inc., Cary NC). No parity effects were observed; therefore, parity was removed from the model. The following model was used to fit the data to assess effects of dietary treatments:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + e_{ijkl},$$

where Y_{ijkl} = dependent variable; μ = overall mean; S_i = effect of square i; P_j = effect of period j; $C_{k(i)}$ = effect of cow k (within square i); T_l = effect of treatment l; ST_{il} = interaction between square i and treatment l; and e_{ijkl} = residual error. All terms were considered fixed, except for $C_{k(i)}$ and e_{ijkl}, which were considered random. The following model was used for ruminal variables, for which repeated measurements over time were used (pH, ammonia, total free AA, RP number, and individual and total VFA):

$$Y_{ijklm} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl} + Z_m + ZT_{ml} + \omega_{ijklm},$$

where Y_{ijklm} = dependent variable; μ = overall mean; S_i = effect of square i; P_j = effect of period j; $C_{k(i)}$ = effect of cow k (within square i); T_l = effect of treatment l; ST_{il} = interaction between square i and treatment l; E_{ijkl} = whole plot error; Z_m = effect of time m; ZT_{ml} = interaction between time m and treatment l; and ω_{ijklm} = subplot error. Repeated measures analyses were performed using the SP(POW) structure of SAS. All terms were considered fixed, except for $C_{k(i)}$, E_{ijkl}, and ω_{ijklm}, which were considered random. For all models used, the interaction term ST_{il} or ST_{im} was removed when $P > 0.25$. Original RP data was tested in SAS for homogeneity of variance and normality; moreover, RP residuals were tested for normality. Log-transformed RP data only marginally improved normality; therefore, analysis was done using original data. The Tukey-Kramer method was used to adjust for multiple comparisons and to perform mean separations (SAS). For all analyses, significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the current study, no differences in DMI (P = 0.18) were observed among treatments (Table 3). Effects of La on DMI have been conflicting. Faciola et

al. (2013) did not observe a reduction in DMI when a single dose of 160 g/d of La was added directly into the rumen just before feeding in a preliminary trial (n = 6) or when up to 243 g/d of La was mixed in the TMR of dairy cows in a larger study (n = 52). Moreover, Faciola and Broderick (2013) observed no reduction in DMI when dairy cows consumed 438 g/d of La in 1 trial; however, in subsequent studies, these authors detected reduced DMI when 220 g/d of La and 404 and 543 g/d of La were fed in the TMR. Others have also observed variable DMI responses to feeding La; Hristov et al. (2009) did not observe reduced DMI when 240 g/d of La was dosed into the rumen of dairy cows before feeding. However, Hristov et al. (2011) later found that DMI was reduced 26% when 240 g/d of La was dosed into the rumen of dairy cows before feeding (26.9 vs. 20.0 kg/d with control and La, respectively). Delivery method, La concentration in the diet, La dosage, and basal diet characteristics all appear to play important roles in the DMI response to La. Hristov et al. (2011) speculated that La may have low palatability and that this may be an issue in its practical usage for suppressing RP. Effects of CO on DMI have also been conflicting. Hristov et al. (2009) saw no reduction in DMI when 240 g/d of CO was dosed into the rumen of dairy cows before feeding. However, Lee et al. (2011) observed reduced DMI when 500 g/d of CO was consumed by dairy cows. However, Jordan et al. (2006) did not find reduced DMI when 41 beef heifers were fed 250 g/d of CO. More recently, Reveneau et al. (2012) reported a 4.2 kg/d reduction in DMI when 5% CO was added to the diets of 6 lactating dairy cows. Hollmann and Beede (2012) also reported reductions in DMI when CO replaced mainly ground corn in the diets of lactating dairy cows, which increased the ether extract content of the CO diet compared with the control (10.4 vs. 5.7%, respectively). Moreover, these authors stated that the high starch levels fed in their diets (30.1%, DM basis) may have led to higher propionate availability, which may have exacerbated the DMI depression observed when CO was fed. The negative effects of CO on DMI reported when CO replaces dietary carbohydrates may be a consequence of higher fat concentration in the diet. Replacing carbohydrates with fats has been associated with DMI depression (Onetti et al., 2003) and some of the possible explanations for this depression in DMI may be related to decreased NDF digestion, oxidation of metabolic fuels, gut peptide responses, and palatability, as explained by Hollmann and Beede (2012).

Dosing La straight into the rumen via rumen cannula theoretically eliminates palatability issues, which may partially explain why some studies did not detect reductions in DMI when dosing La or CO (Hristov et al., 2009; Faciola et al., 2013). Caution should be used when comparing doses among experiments because different amounts of DMI may dilute or concentrate the amount of La or CO actually reaching the rumen. Other important issues include concentrations of total fat, starch, and fiber in the basal diet, all of which may influence DMI response relative to La and CO inclusion (Hristov et al., 2011; Faciola and Broderick, 2013). In

Table 3. Effects of feeding lauric acid (La) or coconut oil (CO) on DMI, milk production and composition, and blood urea concentration[1]

	Diet				
Item	Control	La	CO	SEM	*P*-value
La intake, g/d	0	288	275	—	—
DMI, kg/d	22.5	22.2	22.9	0.6	0.18
Milk yield, kg/d	35.6^{a}	34.1^{b}	35.9^{a}	1.7	<0.01
3.5% FCM,[2] kg/d	39.5^{a}	36.1^{b}	38.6^{a}	1.3	<0.01
3.5% FCM/DMI	1.75^{a}	1.62^{c}	1.68^{b}	0.04	0.02
Milk N/N intake, %	28.7^{a}	28.1^{b}	28.7^{a}	0.4	0.03
Fat, %	4.19^{a}	3.95^{b}	4.05^{ab}	0.14	0.05
Fat yield, kg/d	1.49^{a}	1.32^{b}	1.42^{a}	0.07	<0.01
True protein, %	2.98	3.03	3.01	0.07	0.32
True protein yield, kg/d	1.06^{a}	1.03^{b}	1.08^{a}	0.03	0.03
Lactose, %	4.93^{a}	4.82^{b}	4.77^{c}	0.05	0.05
Lactose yield, kg/d	1.75^{a}	1.64^{b}	1.71^{a}	0.07	<0.01
SNF, %	8.81^{a}	8.76^{ab}	8.69^{b}	0.11	0.05
SNF yield, kg/d	3.14^{a}	3.00^{b}	3.12^{a}	0.11	<0.01
MUN, mg/dL	11.3^{a}	11.1^{a}	10.4^{b}	0.40	<0.01
BUN, mg/dL	13.5^{a}	13.2^{a}	12.2^{b}	0.42	<0.01
BW change, kg/d	+0.04	+0.07	+0.02	0.18	0.34

$^{a-c}$Least squares means within the same row with different superscripts differ ($P < 0.05$).

[1]Data from 30 lactating cows.

[2]FCM = 0.4318 × milk yield + 16.23 × fat yield (Sklan et al., 1992).

the present study, the feeding about 10% dry molasses (DM basis) may have helped to alleviate some of the negative effects of La and CO on DMI (Broderick and Radloff, 2004); therefore, potential palatability problems may have been minimized. Moreover, the La and CO diets were balanced to contain equal amounts of La, and control and CO diets were balanced to contain the same amounts of total fat. This design likely reduced the chances of detecting differences in DMI. Discrepancies in DMI among earlier studies may have arisen from replacing carbohydrates with La and CO or from feeding diets with different fat contents (Hollmann and Beede, 2012).

Milk and 3.5% FCM yields were reduced ($P < 0.01$) when La, but not CO, was fed (Table 3). Compared with the control treatment, La reduced efficiency of milk production, expressed as FCM/DMI ($P = 0.02$), and efficiency of dietary N utilization, expressed by milk N/N intake ($P = 0.03$). Lauric acid also reduced (Table 3) yields of fat ($P < 0.01$), protein ($P = 0.03$), lactose ($P < 0.01$), SNF ($P < 0.01$), as well as milk fat percentage ($P = 0.05$), which may be related to depressed fiber digestion, which will be discussed in more detail herein. Coconut oil reduced FCM/DMI ($P = 0.02$) and concentrations of lactose and SNF ($P = 0.05$) (Table 3). Reductions in yield of milk and milk components were reported previously when La was directly dosed into the rumen (Hristov et al., 2011) and when mixed into the TMR of lactating cows (Faciola and Broderick, 2013); in both studies, DMI depression likely played a role in reducing milk production. Milk yield was not affected in studies in which DMI was not reduced by La (Hristov et al., 2009; Faciola et al., 2013). Hristov et al. (2009) did not observe decreased milk yield when 530 g/d of CO was dosed into the rumen before feeding; however, Lee et al. (2011) reported decreased yield of milk and milk components when 500 g/d of CO was fed to dairy cows. Moreover, Storry et al. (1974) reported depressed yield of milk and milk components when CO was mixed with the concentrate at rates of 10 and 15% (CO intakes ranging from 730 to 1,245 g/d). Reveneau et al. (2012) and Hollmann and Beede (2012) also observed reductions in milk yield when CO was added to the diets of lactating dairy cows and reduced DMI was reported to be the primary reason why milk production was decreased.

Typically, reduced BUN and MUN concentrations indicate improved N efficiency (Broderick and Clayton, 1997; Nousiainen et al., 2004). In the present trial, CO reduced ($P < 0.01$) both BUN and MUN (Table 3), which suggested a more efficient utilization of dietary N for milk protein secretion. However, no differences between the CO treatment and control were detected in either milk protein yield or in milk N/N intake (Table 3). In the present trial, no differences in BW change were noted across treatments (Table 3). Perhaps improved N utilization, as indicated by reductions in BUN and MUN observed in the current experiment, was insufficient to allow higher milk protein production. In agreement with these findings, Lee et al. (2011) have shown similar reductions in MUN and BUN, but no changes in BW with La supplementation.

A decrease of about 40% in RP population ($P < 0.01$) was observed when cows ingested 291 g/d of La or 690 g/d of CO, which provided 276 g/d of La (Table 4). Similarly, Faciola et al. (2013) observed a decrease of 32% in RP numbers when 243 g/d of La was ingested by dairy cows. Faciola and Broderick (2013) observed reductions of 37 and 67% when 129 and 270 g/d of La were consumed, respectively (experiment 1), and reductions of 28 and 49% when 220 and 404 g/d of La were consumed, respectively (experiment 2). The greater antiprotozoal effect of La observed in experiment 1 was probably a consequence of lower DMI in that trial, which would have concentrated La within the rumen. Reduction of RP numbers by La and CO has been reported consistently. Hristov et al. (2009) observed an 80% reduction in RP numbers when 240 g/d of La or 530 g/d of CO were dosed directly into the rumen of dairy cows immediately before feeding. Furthermore, Hristov et al. (2011) observed a 96% reduction in RP numbers when 240 g/d of La was dosed into the rumen of dairy cows before feeding. Faciola et al. (2013) observed a 90% reduction in RP numbers when 160 g/d of La was dosed into the rumen of dairy cows before feeding. Lee et al. (2011) observed a 60% reduction in RP numbers when 500 g/d of CO was consumed mixed in the TMR of dairy cows. Reveneau et al. (2012) reported a 90% reduction in RP population when 5% CO was added to the diet of lactating dairy cows. These results again indicate that delivery method and dose of La and CO both play a role in the degree of RP population suppression.

Ruminal ammonia concentration was reduced when CO and La were fed ($P < 0.01$), whereas total free AA concentration was only reduced by La consumption ($P < 0.01$; Table 4). One of the most common findings associated with reduced RP population has been decreased ruminal ammonia (Williams and Coleman, 1992; Jouany, 1996). Faciola and Broderick (2013) reported reductions in both ruminal ammonia and total free AA concentration when La was consumed by dairy cows. Lee et al. (2011) found a trend toward reducing ruminal ammonia concentration and no change on TAA concentration when the RP population was suppressed 60% with the feeding of 500 g/d of CO to dairy cows. Ruminal protozoa possess protease (Forsberg et al., 1984), peptidase (Newbold et al., 1989), and de-

Table 4. Effects of feeding lauric acid (La) or coconut oil (CO) on ruminal traits[1]

Item	Diet			SEM	*P*-value
	Control	La	CO		
La intake, g/d	0	291	276	—	—
pH	6.16	6.11	6.17	0.07	0.34
Total VFA, m*M*	127.5[a]	122.8[b]	123.5[b]	3.8	<0.01
Acetate, % of total VFA	65.0[a]	63.7[b]	62.6[b]	1.1	0.05
Propionate, % of total VFA	19.5[b]	20.4[b]	22.0[a]	1.3	0.05
Acetate:propionate	3.3[a]	3.1[b]	2.8[c]	0.1	<0.01
Butyrate, % of total VFA	11.7	11.6	11.3	0.6	0.11
Valerate, % of total VFA	1.75[b]	1.84[ab]	1.94[a]	0.15	0.04
Isovalerate, % of total VFA	1.71[a]	1.61[b]	1.66[ab]	0.08	0.05
Isobutyrate + 2-methylbutyrate, % of total VFA	1.07[a]	0.96[b]	0.94[b]	0.09	<0.01
Branched-chain VFA, % of total VFA	2.78[a]	2.57[b]	2.60[b]	0.13	<0.01
Ammonia, m*M*	10.4[a]	9.1[c]	9.6[b]	0.4	<0.01
Total free AA, m*M*	5.2[a]	4.2[b]	5.3[a]	0.5	<0.01
Protozoa, $\times 10^6$ cells/mL	5.71[a]	3.45[b]	3.29[b]	0.21	<0.01

[a–c]Least squares means within the same row with different superscripts differ ($P < 0.05$).

[1]Data from the 6 ruminally cannulated cows.

aminase (Itabashi and Kandatsu, 1975) activities. Hino and Russell (1987) reported that deaminase activity in protozoal extracts was 2 to 3 times higher than in bacterial extracts; therefore, RP appear to degrade dietary protein, peptides and AA. Moreover, protozoa briskly consume bacteria (Coleman, 1975) and in vitro studies indicated that they are the main contributors to bacterial protein turnover in rumen fluid (Wallace and McPherson, 1987). These observations help to explain why suppression of RP population often reduces ruminal ammonia concentration.

Ruminal pH was not affected ($P = 0.34$) by La or CO feeding in this trial, which agrees with previous observations of Faciola and Broderick (2013). In the present trial, ruminal total VFA concentration was reduced ($P < 0.01$) when La and CO were consumed (Table 4), but a reduction from 128 to 123 m*M* probably is of marginal biological significance.

Ruminal propionate molar proportion increased ($P = 0.05$) and acetate-to-propionate ratio decreased ($P < 0.01$) when CO was consumed compared with both control and La treatments (Table 4). Molar proportion of acetate also was reduced by La ($P = 0.05$). Similar results were reported by Storry et al. (1974) and Lee et al. (2011). These results indicated that partial RP suppression leads to greater propionate and lower acetate proportions in ruminal VFA, which may be explained by the lower NDF digestion observed with RP suppression and also by the observation that RP produce predominantly acetate (Williams and Coleman, 1992). Ruminal isobutyrate molar proportion was reduced ($P < 0.01$) by both La and CO; moreover, La reduced ruminal isovalerate plus 2-methylbutyrate molar proportion ($P = 0.05$) (Table 4). These branched-chain VFA (**BCVFA**) are formed in the rumen by deamination of branched-chain AA and concentrations are related to ruminal degradation of dietary protein (Van Soest, 1994). Literature data on the effects of RP suppression on BCVFA are scarce and, because of their very low molar concentration and analytical variations, it has been often difficult to detect statistical differences. Hristov et al. (2011) also reported reduced ruminal isobutyrate concentration when La was dosed directly into the rumen before feeding. Suppression of RP, which has been commonly shown to reduce proteolysis, deamination, and microbial recycling (Wallace et al., 1987; Broderick et al., 1991; Ushida et al., 1991), is likely to reduce BCVFA, and lower BCVFA concentrations may also reflect lower microbial turnover in the rumen. Because of the inconsistencies observed in individual VFA molar proportions reported in RP suppression studies, Veira (1986) suggested that RP per se may not be solely responsible for the observed differences but rather that changes in the bacterial population, often not measured, probably also play a role in VFA pattern and concentration.

In the present study, apparent digestibility of NDF and ADF was reduced ($P < 0.01$) when La, but not CO, was fed (Table 5). Reduction in apparent NDF digestibility after La treatment has been observed (Faciola and Broderick, 2013; Faciola et al., 2013); moreover, similar results have recently been reported after CO feeding (Lee et al., 2011; Hollmann and Beede, 2012; Reveneau et al., 2012). Reduction in NDF digestibility appears to be an effect frequently reported in RP suppression studies (Williams and Coleman, 1992). This could be a result of several factors. Rumen protozoa have been shown to possess cellulase (Hungate, 1966), hemicellulase (Williams and Coleman, 1985), and pectinase (Orpin and Hall, 1983; Orpin, 1984) activities,

Table 5. Effects of feeding lauric acid (La) or coconut oil (CO) on apparent digestibility and N excretion[1]

Item	Diet			SEM	*P*-value
	Control	La	CO		
La intake, g/d	0	288	275	—	—
Apparent digestibility, %					
DM	67.3	66.4	67.2	1.0	0.13
OM	69.4	68.5	69.5	1.0	0.13
NDF	44.4[a]	41.9[b]	44.3[a]	1.0	<0.01
ADF	49.3[a]	46.1[b]	48.5[a]	1.0	<0.01
CP	62.7	63.8	63.9	1.1	0.12
Excretion					
Urine volume,[2] L/d	22.5	23.1	21.8	1.2	0.21
Urinary urea-N, g/d	136	138	131	3.2	0.16
Total urinary N, g/d	166	164	161	3.1	0.23
Urea-N/total urinary-N, %	82.0	84.4	81.2	2.7	0.17
Allantoin, mmol/d	400	396	408	7.1	0.32
Uric acid, mmol/d	35.4	35.2	35.5	4.2	0.28
Purine derivative,[3] mmol/d	435.4	431.2	443.5	15.1	0.16
Fecal N/urinary N	1.30	1.27	1.32	0.26	0.15
Milk N/fecal + urinary N	0.43	0.42	0.44	0.03	0.13
Microbial N flow,[4] g/d	249	246	255	17.0	0.23
Apparent N balance,[5] g/d	46	58	67	16.0	0.11

[a,b]Least squares means within the same row with different superscripts differ ($P < 0.05$).

[1]Data from 30 lactating cows.

[2]Estimated from creatinine concentration in spot urine samples assuming an excretion of 29 mg of creatinine/kg of BW (Valadares et al., 1999).

[3]Allantoin plus uric acid.

[4]Estimated from urinary excretion of purine derivatives according to Valadares et al. (1999).

[5]N intake – (urinary N + fecal N + milk N).

which may explain their role in NDF digestion. Rumen protozoa may also affect the extent of fiber fermentation. Hristov et al. (2004) reported a decrease in carboxymethylcellulase, a measure of total fibrolytic activity, when RP was suppressed by 91% of the control diet. Reductions in NDF and ADF digestion observed when La was fed may explain the reduction of the molar proportion of acetate (Table 4). Because acetate is a major precursor for FA synthesis in ruminants, its reduction may help explain the lower milk fat percentage observed when La was consumed.

No differences in ruminal microbial N flow were observed among treatments when estimated from urinary excretion of purine derivatives (Valadares et al.,1999; Table 5) or when it was measured from ^{15}N enrichment of microbial NAN at the omasal canal. Ruminal microbial N flow determined using purine derivative excretion underestimated microbial N flow by about 30% when compared with omasal flow measured using ^{15}N as a microbial marker, a result that agrees with the observations of Reynal et al. (2005). Among possible reasons for these differences could be (1) physiological differences between the animals used in the present experiment and those used to develop the relationships between purine derivative excretion and ruminal purine outflow, and (2) inaccuracies in spot urine sample collections.

A trend ($P = 0.06$) was noted for reduced omasal DM flow when La was fed (Table 6); however, no differences in DM apparently digested in the rumen and no differences in OM omasal flow or OM digestion in the rumen were observed when either La or CO were consumed. Lauric acid reduced NDF and ADF apparently digested in the rumen ($P < 0.01$) and increased the flow of NDF ($P = 0.05$) at the omasal canal. Data on omasal flow and apparent ruminal digestion of DM, OM, NDF, and ADF with La feeding has not, to our knowledge, been previously reported in the scientific literature. Ruminal fiber digestion accounts for the major portion of the total tract digestion (Ahvenjärvi et al., 2000). Feeding La was shown to decrease total tract fiber digestion in the present study, as presented earlier but also in other studies (Faciola and Broderick, 2013; Faciola et al., 2013); therefore, reduced ruminal fiber digestion would be expected to be with associated with reduced protozoal population in the rumen. Reveneau et al. (2012) reported numerically but not significantly different ruminal NDF digestion when feeding CO and using $YbCl_3$ as a single solid marker (labeled corn silage), Co-EDTA as the liquid marker, and dosing markers 3 times daily as opposed to the continuous infusion applied in the present study. Thus, different marker methodology used may explain some of these differences.

Table 6. Effects of feeding lauric acid (La) or coconut oil (CO) on intake, flow at the omasal canal, and ruminal digestibility of DM, OM, NDF, and ADF[1]

	Diet				
Item	Control	La	CO	SEM	*P*-value
La intake, g/d	0	291	276	—	—
DM					
Intake, kg/d	22.8	22.4	23.0	1.0	0.14
Flow at the omasal canal, kg/d	14.1	13.6	14.2	0.7	0.06
Apparently digested in the rumen, kg/d	8.68	8.77	8.76	0.82	0.21
% of DMI	38.1	39.1	38.1	2.5	0.18
OM					
Intake, kg/d	21.2	20.8	21.4	0.93	0.08
Flow at omasal canal, kg/d	11.4	11.1	11.5	0.62	0.18
Apparently digested in the rumen, kg/d	9.78	9.77	9.88	0.42	0.17
% of OM intake	46.1	46.9	46.2	1.1	0.16
Truly digested in the rumen, kg/d	13.5	13.4	13.6	0.71	0.19
% of OM intake	63.6	64.4	63.7	2.4	0.21
NDF					
Intake, kg/d	6.48	6.43	6.53	0.25	0.16
Flow at the omasal canal, kg/d	4.61[b]	4.82[a]	4.64[ab]	0.19	0.05
Apparently digested in the rumen, kg/d	1.87[a]	1.61[b]	1.89[a]	0.16	<0.01
% of NDF intake	28.8[a]	25.0[b]	29.0[a]	2.1	<0.01
ADF					
Intake, kg/d	4.20	4.12	4.23	0.17	0.15
Flow at the omasal canal, kg/d	2.96	3.08	3.01	0.13	0.07
Apparently digested in the rumen, kg/d	1.24[a]	1.04[b]	1.22[a]	0.10	<0.01
% of ADF intake	29.4[a]	25.2[b]	28.9[a]	2.3	<0.01

[a,b]Least squares means within the same row with different superscripts differ ($P < 0.05$).

[1]Data from the 6 ruminally cannulated cows.

Trends for increased N truly digested in the rumen ($P = 0.07$) and decreased RUP flow at the omasal canal ($P = 0.08$) were observed when CO was consumed (Table 7), which may be a reflection of the trend toward increased RDP supply ($P = 0.09$) on the CO treatment. Diets were formulated to provide similar amounts of RDP and RUP, and, although not significant, small numerical differences in DMI, and therefore N intake, may explain the observed trends in RDP supply and RUP flow. Increased RUP flow previously reported with RP suppression (Jouany, 1996) was not observed in the present study; 40% suppression in RP population may not have been enough to promote this effect. No differences were observed in NAN flowing with fluid- and particle-associated bacteria in the current study (Table 7). It is not clearly understood how suppressing RP would affect different types of bacteria. Studies reporting differences in the bacterial community were often done in vitro and with complete elimination of the RP population (Ushida et al., 1987), conditions very different from the current study. In our study, suppression of 40% of RP population did not change total microbial NAN flow and microbial efficiency, measured as microbial NAN per unit of OM truly digested in the rumen (Table 7). Ivan (2009) observed decreased total NAN and bacterial NAN flow when RP were inoculated into RP-free sheep, which suggests a beneficial effect of suppressing RP in vivo. However, achieving such effects in a practical setting without the negative effects of various RP-suppressant agents on DMI, ruminal fermentation, and nutrient and fiber digestion remains a challenge.

CONCLUSIONS

Results from our experiment have confirmed that dietary La is not practical for suppressing RP population in lactating dairy cows, mainly because of its negative effects on fiber digestion, ruminal fermentation, and, consequently, milk production. Consumption of CO at 687 g/d or La at 288 g/d mixed in the TMR did not reduce DMI of lactating dairy cows. However, feeding La reduced yields of milk, 3.5% FCM, fat, protein, lactose, and SNF. Lauric acid also reduced the efficiency of conversion of energy and N into milk. Despite not affecting DMI, reducing ruminal ammonia, MUN, and BUN concentrations, and increasing molar proportions of propionate, feeding CO reduced efficiency of conversion of feed energy into milk, did not improve yield of milk and milk components, and did not increase microbial synthesis in the rumen. Lauric acid markedly reduced ruminal and total tract apparent digestibility of NDF and ADF. Both La and CO reduced ruminal total VFA concentration, molar proportions of acetate, and acetate-to-propionate ratio. Lauric acid consumption increased NDF flow at the omasal canal. Both CO

Table 7. Effects of feeding lauric acid (La) or coconut oil (CO) on intake and flow of N fractions at the omasal canal[1]

	Diet				
Item[2]	Control	La	CO	SEM	*P*-value
La intake, g/d	0	291	276	—	—
Dietary N intake, g/d	587	581	592	14	0.11
Omasal flows					
Total NAN, g/d	523	522	525	25	0.19
NAN[2] % of N intake	89.1	89.9	88.7	2.1	0.16
N truly digested in the rumen, g/d	414	404	423	23	0.07
RDP supply					
kg/d	2.52	2.46	2.58	0.15	0.09
% of diet CP	68.7	67.7	69.6	2.2	0.08
% of DMI	11.0	10.9	11.2	0.4	0.06
RUP flow					
kg/d	1.15	1.17	1.13	0.06	0.08
% of diet CP	31.3	32.3	30.4	2.1	0.07
% of DMI	5.0	5.2	4.9	0.5	0.09
NMNAN flow					
g/d	173	176	169	15	0.16
% of total NAN	33.1	33.8	32.2	1.8	0.08
% of N intake	29.5	30.4	28.5	2.1	0.08
Microbial NAN flows					
FAB-NAN					
g/d	152	149	155	10	0.13
% of microbial-NAN	43.4	43.2	43.5	1.8	0.32
PAB-NAN					
g/d	198	196	201	16	0.16
% of microbial-NAN	56.6	56.8	56.5	1.7	0.23
Total microbial NAN					
g/d	350	345	356	19	0.12
% of total NAN	66.9	66.2	67.8	1.9	0.11
Microbial efficiency, g of NAN/kg of OMTDR	26.0	25.7	26.1	0.7	0.12

[1]Data from 6 ruminally cannulated cows.

[2]NMNAN = nonmicrobial NAN; FAB- and PAB-NAN = fluid- and particle-associated bacterial NAN; OMTDR = OM truly digested in the rumen.

and La reduced RP numbers by about 40%; therefore, based on the results of our study, a 40% reduction of RP population is not sufficient to improve N utilization in dairy cows.

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